the selectivity filter, conserved in both structure and sequence, presents a series of backbone carbonyls towards the permeating ion. Here, we have performed a full three-ion permeation free energy landscape calculation for the selectivity filter region using all-atom umbrella sampling molecular dynamics simulations of the KcsA channel in a membrane with explicit lipids and solvent. The cases of pure potassium conduction and of a single sodium chaperoned by potassium ions were examined. The Potential of Mean Force (PMF) dependent on the Z-coordinates of three ions was calculated on the basis of a new unpublished high-resolution crystal structure of KcsA in a conformation which includes both an open gate and a conductive filter. The effect of flipping of Valine 76 was examined as well as the permeation and selectivity in a model with Glycine 77 replaced by a D-Alanine.

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Ion Permeation in the MthK Potassium Channel

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KcsA from S.lividans is well established as an archetype model for the whole family of potassium channels. However, MthK from M. thermoautotrophicum, another model potassium channel, behaves quite differently in many respects. Comparison of functional and structural data of both channels reveals important similitudes, but also key differences. An important but not well understood feature of potassium channels is their C-type inactivation mechanism. The structure of the inactivated state of KcsA was first solved by crystallization under conditions of low potassium concentration. It revealed conformational changes in the selectivity filter region preventing the permeation of both K(+) and Na(+) ions. Surprisingly, x-ray crystallography experiments under similar low potassium conditions revealed that the structure of MthK remains in its canonical conducting state even when it is deprived of K(+) ions. Functional studies nevertheless show that MthK undergoes C-type inactivation like KcsA and most eukaryotic K(+) channels. To understand the mechanisms underlying this apparent discrepancy, we investigated on the permeation and gating mechanisms in the MthK channel using molecular dynamic simulations and free energy calculations.

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Mg^{2+} Blockade in a Kv Potassium Channel Mutant having an Unusually High Conductance

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Voltage gated K-channels (Kv) family members are endowed of a highly K^+ selective pore and diverse single channel conductances, ranging from 3 to 300 pS in ~100 mM K^+. The selectivity filter is very conserved among K-channels, thus, the origin of such diversity must reside in other aspects of the pore. A Shaker Kv channel having a point mutantion at the internal entrance (Pro475Asp) conducts single channel currents 6-8 fold larger than the wild type channel (WT). This variant also showed ~100-fold increased sensitivity for intracellular Mg^{2+}. Near zero-voltage Mg^{2+} binding was antagonized by K^+ in a conventional competitive fashion. However, the voltage dependence of Mg^{2+} blockade increased substantially with K^+ concentration, with a effective valence being near zero in symmetrical 50 mM K^+ to ~0.4 at 500 mM K^+, not consistent with a competitive scheme at voltages away from zero. This K^+ enhanced voltage dependence is consistent with a pore being narrow enough to sustain single file diffusion in which K^+ could occlude the Mg^{2+} exit towards the cytosolic face of the mutant channel. Molecular dynamics simulations of Shaker WT and P475D reveal dramatically increased density of K^+ ions near 475D side chains, while K+ occupancy at the selectivity filter remains unchanged. In agreement with the observed K-enhanced voltage dependency, ion density profile of both K^+ and Mg^{2+} in the pore, showed K^+ occupation of a more distal binding site than that of Mg^{2+}. The enhanced apparent affinity for Mg^{2+} could stem from this lock-in K^+ binding site located at the internal entrance of the pore. Larger single channel conductance in this mutant could be supported by an increased occupancy of a pore long enough to hold several cations in single file.

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hERG Quality Control at the Plasma Membrane

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The KCNH2 or human ether-a-go-go related gene (HERG1) encodes the Kv11.1 protein that is responsible for the rapidly activating delayed rectifier K+ current (IKr). Mutations of HERG cause prolongation of cardiac ventricular repolarzation that underlies the long QT syndrome (LQT2) and associated risk of sudden death. Genetic analyses have identified over 200 LQT2-associated mutations the majority of which impair the exit of HERG from the endoplasmic reticulum (ER). While it is possible to rescue the trafficking, the fate of mutant HERG in post-ER compartments and specifically at the plasma membrane remains elusive. To address this we evaluated the peripheral stability of mutant HERG partially rescued by low temperature (26°C). Temperature-rescued mutant HERG (F805C and G601S), detected by immunoblotting, was eliminated more rapidly than wt HERG upon inhibiting translation with cyclohexamide. The increased plasma membrane density of both F805C and G601S following temperature rescue was demonstrated by cell surface ELISA assay. However, the mutant channels were found to be less stable that the wt HERG due to accelerated internalization and preferential lysosomal sorting. Endosomal sorting of FITC-tagged HERG demonstrates that wt HERG was confined to a pH compartment characteristic of recycling endosomes whereas mutant HERG were delivered to vesicles with luminal pH typical of late endosomes and lysosomes. Using ts20 and E36 CHO cells we showed that temperature-induced inactivation of the E1 enzyme delayed the delivery of F805C and G601S HERG into lysosomes. Downregulation of Hrs, Stam1 and TSG101 ubiquitin-binding adaptors of the ESCRT0-I by siRNA also induced early endosomal retention in a compartment with a luminal pH of sorting/recycling endosomes. These results indicate the involvement of an ubiquitin-dependent peripheral HERG quality control system that accelerates the internalization of mutant HERG and targets them for lysosomal degradation.

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Effect of the R1047L AF/LQT Mutation on the Distal C-Terminal Domain of the hERG Potassium Channel

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Kv11.1 (hERG, KCNH2) is a voltage-gated potassium ion channel subunit involved in repolarization of cardiac action potential by mediating IKr current. Loss-of-function mutation or blockade of the channel pore by drug can induce a potentially fatal disorder called long QT syndrome (LQT). Atrial fibrillation (AF), the most commonly treated arrhythmia, has been linked to ion channel dysfunction. A significant proportion of AF patients have a positive family history and may therefore be affected by inherited mutations. We have studied gene variations in the C-terminal domain (CTD) of hERG isolated from patients with AF. One such variant, R1047L, is known to be associated with both AF and LQT, but its effects on the structure, stability, and function of hERG remain to be studied. Previous studies have shown that this site is within a coiled-coil domain that is thought to promote subunit assembly and form protein-protein interfaces. Electrophysiological studies showed difference in the tail current profile for wild-type (WT), R1047L, and the R1047L-WT heteromer. For structural studies, we made constructs of wt and R1047L mutant distal CTDs and purified with isotope labeling for high resolution NMR spectroscopy. 1H-15N HSQC spectrum of the WT distal CTD showed that the purified domain is consistent with predictions based on sequence analysis. Circular dichroism (CD) spectroscopy showed this domain is largely helical. Size-exclusion chromatography (SEC) and multi-angle light scattering (MALS) confirmed its molecular weight corresponds to a tetramer, as is expected for coiled-coil domains in voltage-gated ion channels. A comparison is presented of the stability and structural properties of the WT and R1047L mutant forms of this domain.